CYTOLOGICAL FEATURES OF "NON-INVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES" AND THEIR CORRELATION WITH TUMOR HISTOLOGY

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SHORT TITLE: FNAB cytology features of NIFTP of the thyroid

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ABSTRACT

Among thyroid papillary carcinomas (PTC), the follicular variant is the most common and includes encapsulated forms (EFVPTC). Non-invasive EFVPTC have very low risk of tumor recurrence or other adverse events and have been recently proposed to be designated as “Non-invasive follicular thyroid neoplasm with papillary-like nuclear features” or “NIFTP”, thus eliminating the term “carcinoma”. This proposal is expected to significantly impact the risk of malignancy associated with the diagnostic categories currently used to report thyroid cytology. In this study, we analyzed the FNAB cytology features of 96 histologically proven NIFTP and determine how the main nuclear features of NIFTP correlate between cytologic and histologic samples. Blind review of FNAB cytology from NIFTP nodules yielded the diagnosis of “follicular neoplasm” (Bethesda category IV) in 56% of cases, “suspicious for malignancy” (category V) in 27%, “atypia of undetermined significance/follicular lesion of undetermined significance” (category III) in 15%, and “malignant” (category VI) in 2%. We found good correlation (k=0.62) of nuclear features between histological and cytological specimens. Nuclear features (size, irregularities of contours and chromatin clearing) of NIFTP were significantly different from those of benign nodules, but not from those of invasive EFVPTC. Our data indicate that most of NIFTP nodules yield an indeterminate cytological diagnosis in FNAB cytology and nuclear features found in cytology samples are reproducibly identified in corresponding histology samples. Because of the overlapping nuclear features with invasive EFVPTC, NIFTP cannot be reliably diagnosed preoperatively, but should be listed in differential diagnosis of all indeterminate categories of thyroid cytology.

KEY WORDS: thyroid cancer, thyroid nodules, follicular variant papillary carcinoma, encapsulated, cytology, NIFTP

INTRODUCTION

Cancer of the thyroid gland is the most common endocrine malignant neoplasm, and papillary thyroid carcinoma (PTC) accounts for the vast majority of cases. Generally, PTC has a favorable prognosis, with long-term survival rates in excess of 95% [1]. It is well documented that the substantial increase of the incidence of thyroid cancer is mainly due to early detection of neoplasms with indolent behavior, mainly small cancers (microcarcinomas) or the use of more relaxed histopathologic criteria for the follicular variant of PTC (FVPTC) [2, 3].

In particular, the encapsulated form of FVPTC (EFVPTC) [4] accounts for 10-20% of all thyroid cancers currently diagnosed in Europe and North America [5, 6]. The pathological diagnosis of this variant is associated with a high degree of inter-observer variability and discordance [7, 8], because it is mainly based on the detection of nuclear features of PTC, which are frequently less evident than in classical PTC. The correct recognition of this entity is thus very subjective. In addition, EFVPTC is known to have an indolent behavior [5, 9, 10]. Cases with incomplete evidence of papillary type nuclei have been either assigned to the category of PTC, or downgraded to follicular adenoma or a category of well differentiated tumors of uncertain malignant potential (WDT-UMP), as suggested by some European authors [11-14].

A recent multi-institutional study examined a large cohort of a well-annotated EFVPTC and established that none of 109 patients with non-invasive EFVPTC followed for 10-26 years developed recurrence or other disease manifestations [3]. Based on this information, the international multidisciplinary group of authors recommended to reclassify these tumors as “non-invasive follicular thyroid tumor with papillary-like nuclear features” (NIFTP). NIFTP is defined by a set of reproducible diagnostic criteria that include nuclear features of the papillary carcinoma,

such as nuclear size, nuclear membrane irregularities, as well as chromatin clearing (ground-glass appearance), found in a non-invasive encapsulated follicular-patterned tumor.

Fine needle aspiration biopsy (FNAB) is the most important and reliable diagnostic procedure for pre-operative evaluation of a thyroid nodule. At present, most thyroid FNAB specimens are classified according to the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) which includes six diagnostic categories, each of them corresponding to a different risk of malignancy [15]. Other classification systems are currently in use in Europe, including those proposed by the British Thyroid Society [16] and by the Italian SIAPEC [17], both of them comprising six diagnostic categories, as well. Several previous reports pointed out the challenges in the cytological diagnosis of the FVPTC due to partially overlapping features with both benign and malignant follicular-patterned lesions [14, 18].

Although histologic features of NIFTP have been well characterized [3], how reliably these features can be identified in pre-operative cytology samples remains unknown. Furthermore, it remains to be determined what cytological diagnoses the NIFTP nodules will yield in thyroid FNAB and what will be an impact of reclassification of non-invasive EFVPTC on the risk of malignancy in the categories of the Bethesda system. A recent multicentric study suggested that by excluding non-invasive encapsulated FVPTC, many of which are expected now to be diagnosed as NIFTP, the risk of malignancy will decrease significantly in the three indeterminate cytology categories (Bethesda III through V) [19]. In addition, such reclassification will have a profound impact on patient management, as the lesions classified as NIFTP will not require completion thyroidectomy or post-surgical radioiodine therapy. In this study, we provide a detailed analysis of a large series of NIFTP from three Italian institutions in order to characterize their cytological features and correlate them with histologic outcomes.

MATERIALS AND METHODS

Case selection - Cases of NIFTP were retrospectively collected from six different hospitals of three Italian institutions (Universities of Turin, Bologna and Pisa). Before revision, cyto-histological cases were matched and each slide was anonymized and coded by a pathology staff member not involved in the study. Histological materials filed under the original diagnosis of encapsulated FVPTC, WDT-UMP or follicular adenoma with occasional clear nuclei were examined. Criteria for inclusion were: i) histological diagnosis of NIFTP according to the reported criteria (see below) [3], and ii) availability of an adequate FNAB biopsy sample performed in the same thyroid nodule. In detail, the diagnostic criteria for NIFTP included the follicular growth pattern, complete encapsulation, no invasion, and papillary cancer type nuclei [3]. The latter were assessed by evaluating three nuclear parameters: (a) size and shape (enlargement/overlapping/crowding and elongation), (b) membrane irregularities (irregular contours, grooves and pseudoinclusions), and (c) chromatin characteristics (chromatin clearing with margination, glassy nuclei). Criteria for exclusion were: vascular invasion or extrathyroidal extension, capsular infiltration (or incomplete/ absent capsule) or the presence of a papillary architecture in more than 1% of the tumor. No multifocal tumors were included, with the exception of 2 cases with incidentally found papillary microcarcinoma <5 mm in size. After histologic slide review, 96 cases of NIFTP were identified: an initial set of 55 from the University Hospitals in Turin (20 from Molinette Hospital, 8 from Mauriziano Hospital and 27 from San Luigi Hospital), 21 cases from Bologna (Maggiore and Bellaria Hospitals), and 20 cases from the Santa Chiara Hospital in Pisa. A portion (14/21) of the NIFTP cases from Bologna had already been included in the original histological series of NIFTP cases [3].

For comparison, benign follicular lesions and invasive EFVPTC with adequate FNAB samples were also included. The former group (37 cases from Molinette Hospital) was represented by

microfollicular hyperplastic goiter (19 cases) and conventional (non-oncocytic) follicular adenoma (18). The invasive EFVPTC cases (24 cases from Molinette and Santa Chiara Hospitals in Turin and Pisa, respectively) had vascular or capsular or parenchymal invasion. The 20 cases from Pisa had already been included in the original histological series as EFVPTC with invasion [3].

Case review - All FNAB cytology cases and corresponding surgical specimens were reviewed independently by the local senior pathologists (MP, MV, GT, GC, FB), and all of them were re-screened by two pathologists (FrM, FeM) together with the contributing pathologist from each Institution. The nuclear score was determined, and for each of the three nuclear parameters, a percentage of expression was assigned, reflecting the extent of the abnormality observed (size/ shape, nuclear membrane profile, chromatin appearance). Score 0 was assigned if the single parameter was absent or present in <50% of cells. Score 1 was assigned if the parameter was clearly evident in ≥50% of cells. The sum of the three scores yielded a final value of 0 to 3.

In all cases, alcohol-fixed or air-dried smears, stained with hematoxylin and eosin (H&E) or Papanicolaou or Giemsa were available for evaluation. In addition, in 92 cases, 4 μm-thick sections obtained from alcohol-fixed, paraffin-embedded cell-blocks stained with H&E or Papanicolaou were available for review. FNAB cytological features from 55 histologically confirmed NIFTP and 61 benign or malignant tumors were assessed blindly, i.e. without knowing the histologic diagnoses. The presence/absence of the following parameters was recorded: (i) nuclear enlargement (at least twice the size of a red blood cell); (ii) nuclear membrane abnormalities (irregularities of contour, pseudoinclusions, grooves); (iii) optically clear, ground-glass nuclei; (iv) nuclear molding; (v) exclusive presence of microfollicular pattern (no macrofollicles); (vi) colloid; (vii) prominent nucleoli; (viii) fibrous tissue; (ix) associated macrophages; (x) loosely cohesive cellularity; (xi) tridimensional clusters of follicular cells; (xii) high cellularity. For the first three parameters, the

extent to which each parameter was represented was recorded as a percentage. In order to parallel the evaluations performed on the corresponding histologica l cases, a score 0 was assigned if the single parameter was absent or present in <50% of cells and score 1 if the parameter was present in ≥50% of cells, thus yielding a final sum of the three scores from 0 to 3. In some cases, the percentage of nuclear irregularities could not be reliably assessed due to low cellularity, fixation artifact, poorly-stained or partly obscured follicular cells. In these cases, the cytological score was assessed on the basis of the presence/absence of a single parameter, without considering the 50% cut-off.

CD56 Immunohistochemistry - In a subset of cases, immunocytochemistry for CD56 (clone 123C3, Ventana Medical Systems, Tucson, USA, diluted 1/100) was performed on cell-blocks. For each case, a percentage of positive cells was assessed. Cases with >80% of positive cells were considered diffusely positive, while cases showing a range of positive cells between 1 and 80% were considered focally positive; cases with no positive cells were considered negative [20, 21].

Statistical analysis - Fisher's exact or Chi-square and Student's t tests, for categorical and continuous variables, respectively, were performed to select the cytological parameters associated to the diagnosis of NIFTP, *versus* benign and malignant tumors, to compare nuclear scores between the groups, to correlate nuclear score and Bethesda categorization, and to compare CD56 immunoreactivity in different tumor types.. A test of concordance (Cohen's kappa) was used to calculate the agreement between histological and cytological scores. The correlation between the extent of the nuclear changes in cytological and histological samples was made using a two tailed Spearman's test. Statistical significance was set at a level of 0.05. All analyses were conducted using.SPSS statistical package .

RESULTS

Cytological diagnosis of NIFTP nodules in FNA samples - The results of blind review of cytology FNAB from nodules histologically diagnosed as NIFTP, benign nodules, and invasive EFVPTC are summarized in **Table 1**. Following the Bethesda System for reporting, most (56%) of NIFTP nodules were diagnosed as "suspicious for a follicular neoplasm" (category IV), followed by "suspicious for malignancy" (category V) (27%), and "atypia of undetermined significance/ follicular lesion of undetermined significance" (category III) (15%). Only 2.1% cases were called "malignant" (category VI). On the contrary, benign follicular adenomas and microfollicular hyperplastic nodules were diagnosed as Bethesda category IV (54%) or category III (46%), while invasive EFVPTC were typically diagnosed as Category IV (62.5%) or Category V (37.5%).

Nuclear score evaluation in histological samples - All 55 NIFTP cases in the initial set had a nuclear score of 2 (21 cases) or 3 (34 cases), while benign nodules were scored 0 or 1 and invasive EFVPTC were scored 2 or 3. The mean percentage of nuclear irregularities was significantly different (Student t-test, $P=10^{-xx}$) between NIFTP and benign follicular lesions for each of the three parameters (92.9% *vs* 21.3% for nuclear size, 72.4% *vs* 4.3% for nuclear membrane irregularities and 63.8% *vs* 6.2% for chromatin clearing). Conversely, the differences between NIFTP and invasive EFVPTC were not statistically significant (the latter having mean percentages of 84.2%, 72.9% and 61.7% for enlarged nuclear size, membrane irregularities, and chromatin clearing, respectively).

Nuclear score evaluation in cytological samples - Among all cytological parameters analyzed,

nuclear enlargement, nuclear membrane irregularities, optically clear/ground-glass nuclei and nuclear molding were significantly associated to NIFTP diagnosis, irrespective of being evaluated in smears, cell-blocks or both (**Table 2**). A significantly different mean percentage of nuclear alterations (Student t-test, P=xxx) was found between NIFTP cases and benign follicular lesions for each of the three parameters (63.8% *vs* 33.5% for nuclear size, 69.6% *vs* 10.3% for nuclear membrane irregularities and 53.9% *vs* 10% for chromatin clearing). Conversely, as for histological samples, no significant differences were found in FNAB of NIFTP and invasive EFVPTC (**Table 3**

In NIFTP, FNAB samples most frequently had a nuclear score of 2 (22 cases) or 3 (n 19 cases), whereas benign follicular lesions had nuclear score 0 in 23 cases, score 1 in 12 cases, and score 2 in 2 cases. Invasive EFVPTC showed the nuclear score of 3 or 2. Comparing the distribution of scores (from 0 to 3) a significant difference was found only between NIFTP and follicular lesions (chi-square test $P<0.05$ VALORE ESATTO), but not between NIFTP cases and invasive EFVPTC (chi-square test $P=0.15$).

Cytological-histological correlations - In cytological samples, comparing the nuclear scores grouped as 0-1 vs 2-3 with the Bethesda System categories for NIFTP and other lesions (**Table 4**) a significant correlation was found only for NIFTP (chi-square test $P<0.001$). In the whole series, the degree of agreement between cytological and histological scoring was good (Cohen's kappa= 0.62, 95% confidence interval 0.49-0.74) (**Table 5**). Examples of cytol-histological correlation are shown in **Figure 1**. Comparing paired cytological and histological samples among all lesion types, a significant correlation was found in the recognition of nuclear enlargement (Spearman r= 0.430, $P<0.05$), nuclear membrane irregularities (Spearman r= 0.794, $P<0.05$) and of optically clear nuclei (Spearman r= 0.648, $P<0.05$). A significant, although weaker, correlation was maintained in the restricted group of NIFTP (Spearman r= 0.316, $P<0.05$ for nuclear enlargement; Spearman r=

0.605, $P<0.05$ for nuclear membrane irregularities; Spearman r= 0.401, $P<0.05$ for optically clear nuclei).

CD56 expression_- In FNABs of NIFTP, CD56 had a variable expression, with 20/36 (55%) negative tumors, similar to classical papillary carcinoma. The remaining cases had CD56 expression either diffuse (>80% of cells) in 10/36 (28%) or focal (10 to 25% of cells) in 6 (17%) cases. Benign nodules were CD56 positive either diffusely in 29/36 (80%) cases, or focally (30% of cells) in 2 (6%), and negative in 5 (14%) cases (**Figure 2**). There was significant difference between NIFTP and benign follicular lesions (chi-square test $P<0.001$), supporting the notion that NIFTP has a CD56 expression profile intermediate between follicular adenoma and papillary cancer.

DISCUSSION

In this study, we report for the first time the cytological features and diagnoses for tumors that histologically belong to a recently described entity of "Non-invasive follicular tumor with papillary type nuclei" (NIFTP) [3]. The results of our analysis indicate that despite heterogeneous expression of nuclear features of papillary carcinoma in the NIFTP nodules, a significant correlation of nuclear features is present between histological and cytological samples in the majority of case. Overall, the most common cytologic diagnosis rendered in FNAB of histologically proven NIFTP nodules is "follicular neoplasm" (Bethesda IV). However, a number of cases had sufficiently evident nuclear features to suggest a diagnosis of suspected malignancy, while in single cases the diagnosis of a papillary carcinoma could be rendered.

In histological specimens, NIFTP is defined as a non-invasive, encapsulated follicular-patterned tumor with cytological features of papillary carcinoma. According to the initial description, the

presence of papillary carcinoma type nuclei in NIFTP is heterogeneous in terms of both qualitative and quantitative changes. A nuclear score offered for diagnosing these tumors in the resected specimens is based on the identification of enlarged nuclei, irregular nuclear borders and chromatin clearing [3]. Assigning a value of 1 to each detected parameter, a NIFTP diagnosis requires at least two of the three above features, thus determining a score of 2 or 3. In this study, we find that these features, either all of the three or at least chromatin clearing and irregular borders, can be recognized in FNAB cytology samples, provided that sufficient cellularity is obtained in either smears or cell-blocks or both. More unpredictable is the recognition of such parameters in the NIFTP cases that have focal or only occasional papillary-type nuclei. In those case, the cytological diagnosis Bethesda IV or even III is frequently given.

We found a good correlation between nuclear scores detected in surgical specimens and those in FNAB cytology material. Among specific features, nuclear membrane irregularities had the best correlation. In FNAB cytology specimens, irrespective of being smears or cell-blocks available, the tumor cell size was the least well performing feature, perhaps due to the difficulty of reliably scoring nuclear size in cytology. Of interest, classical features of papillary carcinoma, such as nuclear vacuoles and grooves, were not invariably present in NIFTP. In addition, in some cases of NIFTP, papillary carcinoma-type nuclear features may not be well evident and the co-existence of cell clusters containing follicular cells with dark slightly enlarged nuclei with smooth border prevents the cytologic diagnosis of suspicious for malignancy, and instead prompts diagnosing these samples as “Follicular neoplasm”/Bethesda IV.

Overall, among the three groups of follicular-patterned lesions with known surgical pathology diagnosis studied in this series, most were diagnosed in FNAB samples as either “follicular neoplasm or suspicious for follicular neoplasm” or “atypia of undetermined significance/follicular

lesion of undetermined significance" according to the Bethesda system. One of these two cytologic diagnoses was rendered in 100% of benign follicular adenomas/microfollicular goiter nodules, in 63% of invasive EFVPTC, and in 71% of NIFTP cases. However, about one-fourth of NIFTP were interpreted as "suspicious for malignancy" and two tumors as malignant. When compared with the resected specimen, the two cases diagnosed by cytology as malignant had complete encapsulation with no invasion and a follicular architecture with cells having large, markedly irregular nuclei, diffuse clearing of chromatin and scattered nuclear pseudoinclusions and grooves. The existence of such cases raises a possibility that in a cytological sample that lacks evidence of papillary architecture, the finding of even fully developed PTC nuclei cannot exclude the possibility of NIFTP diagnosis after surgery. Since this new terminology is expected to replace a significant proportion of cases previously classified as EFVPTC, this clinical scenario may be relatively common. In a recent study, the impact of reclassifying non-invasive encapsulated follicular variant papillary carcinomas, now designated as NIFTP, on the risk of cancer predicted by FNAB cytology was investigated [19]. It showed that the risk is expected to be reduced in all categories of indeterminate cytology, including atypia of indeterminate significance, follicular neoplasm, and suspicious for malignancy. The current study confirms this expectation directly. As a result, the changes in the reporting of FNAB and predicting the risk of malignancy in these categories of cytology defined by the Bethesda system diagnostic category will be needed.

Importantly, NIFTP appears to have a very indolent behavior, with the risk of recurrence less than 1% in 10 years, as opposed to invasive encapsulated FVPTC that can metastasize and result in patient death [3, 11]. Since the detection of invasion requires surgical excision, this will likely to remain an appropriate therapeutic approach for these tumors. However, in the absence of invasion, it appears that neither completion of thyroidectomy, nor radioiodine treatment is required.

Among additional diagnostic modalities, molecular tests of FNAB material may be useful to distinguish conventional invasive PTC, which more likely to contain *BRAF* mutations or *RET/PTC* rearrangements, from pure follicular patterned neoplasms with papillary type nuclei, including invasive EFVPTC and NIFTP, which frequently carry *RAS* mutations or *PAX8-PPARG* fusions [3]. However, whether molecular genotyping can provide high sensitivity and specificity of NIFTP diagnosis in FNAB sample remains to be elucidated. CD56, a neural adhesion molecule expressed in different cells and tumors, including endocrine tumors, was recently proposed as a differential marker to separate follicular tumors from PTC, with the latter being negative [21]. CD56 expression was found to be heterogeneous in this series of NIFTP, with an intermediate profile between follicular and papillary cancers, thus supporting the view that NIFTP shares cytomorphological features with PTC, although CD56 loss was not as complete as reported in conventional PTC [21, 22].

In conclusion, in this study we observed good correlation of cytological nuclear features between histological and cytological specimens of NIFTP. Moreover, although this tumor cannot be diagnosed with certainty based on FNAB cytology, we showed that nuclear features of NIFTP are significantly different from those of benign follicular tumors and hyperplastic nodules, but not from those of EFVPTC with invasion. Therefore, the presence of papillary carcinoma-type nuclei in a follicular-patterned nodule observed in FNAB cytology should indicate the possibility of not only PTC, but also NIFTP. This could affect the extent of surgery since patients with NIFTP are at very low risk for unfavorable outcome after the nodule excision by hemithyroidectomy.

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Table legends

Table 1. Clinical data and cytological categorization of FNAB according to the Bethesda System.

Table 2. FNAB cytology parameter assessment in NIFTP and benign follicular lesions
* $P<0.05$ was considered statistically significant.

Table 3. Occurrence of the three most relevant FNAB cytology parameters in NIFTP and other lesions.
NOTE. The *P*-value assesses the differences in the extent of the three parameters between NIFTP and benign follicular lesions (*) and between NIFTP and invasive EFVPTC (**)

Table 4. Correlation between nuclear score and categorization according to the Bethesda System in different tumor types.
NOTE. The chi-square test showed significant correlation between nuclear score and Bethesda categorization in the NIFTP category (Chi-square: 32.999, $P<0.001$)

Table 5. Concordance of scores between FNAB cytology and histology in the whole series of lesions studied.

Figure legends

Figure 1. Illustrations of the nuclear features and nuclear scores in cytological and histological material from a follicular adenoma and two cases of NIFTP. The follicular adenoma shows small nuclei (less than twice the size of a red blood cell) (A, alcohol-fixed smear stained with H&E; x400), without chromatin clearing (B, alcohol-fixed H&E-stained smear; x400) and with round, regular contours (C, air-dried Giemsa-stained smear; x400). The corresponding histological sample (D, H&E; x400) shows a microfollicular lesion with small, dark and round, regular nuclei, not significantly different from the normal thyroid around the nodule (*inset*). The second column exemplifies a case of NIFTP with large (E, air-dried smear stained with Giemsa; x400), but dark nuclei (F, alcohol-fixed Pap-stained smear; x400); anyway, irregularities of nuclear contours with grooves could be appreciated (G, alcohol-fixed Pap-stained smear; x400). The corresponding histological sample (H, H&E; x400) shows large and irregular, but prevalently dark nuclei, if

compared to normal thyroid (*inset*). The third column illustrates a case of NIFTP with nuclear score 3 on both cytological (alcohol-fixed Pap-stained smear in I,L and H&E-stained in M; x400) and histological samples (N, H&E; x400).

Figure 2. CD56 immunostaining results in a case of microfollicular goiter (A,B), showing CD56 intense and diffuse membranous positivity (B) and in a case of NIFTP (C,D), showing a complete loss of expression of CD56 in neoplastic cells. Sections obtained from cell-blocks and stained with H&E (A,C; x400) and antibodies anti-CD56 (B,D; x400).

Table 1. Clinical data and cytological categorization of FNAB according to the Bethesda System

	No.	Age (yrs, median)	Sex		Size (cm, mean)	Cytological categories according to the Bethesda System				
			F	M		II	III (%)	IV (%)	V (%)	VI (%)
NIFTP	96	46	70	26	2.6	0	14 (15)	54 (56)	26 (27)	2 (2)
Benign follicular lesions	37	62	22	15	3.2	0	17 (46)	20 (54)	0	0
Invasive EFVPTC	24	43	15	9	2.2	0	0	15 (62.5)	9 (37.5)	0

Table 2. FNAB cytology parameter assessment in NIFTP and benign follicular lesions

	NIFTP	**Benign follicular lesions**	**Fisher's exact test *P*-value**
Nuclear enlargement	37/55	14/37	0.0098*
Nuclear membrane irregularities	47/55	1/37	0.0001*
Optically clear, ground-glass nuclei	29/55	1/37	0.0001*
Nuclear molding	50/55	20/37	0.0001*
Microfollicular pattern only (no macrofollicles)	48/55	10/37	0.1037
Colloid	7/55	8/37	0.2678
Prominent nucleoli	15/55	17/37	0.077
Fibrous tissue	22/55	21/37	0.1384
Associated macrophages	12/55	11/37	0.4642
Loosely cohesive cellularity	39/55	28/37	0.6422
3D clusters of follicular cells	3/55	2/37	1
High cellularity	15/55	10/37	1

* $P<0.05$ was considered statistically significant.

Table 3. Occurrence of the three most relevant FNAB cytology parameters in NIFTP and other lesions

	N.	**Nuclear size**		**Nuclear membrane irregularities**		**Chromatin clearing**	
		mean	**range**	**mean**	**range**	**mean**	**range**
NIFTP	55	63.8	0-100	69.6	0-100	53.9	0-100
Benign follicular lesions	37	33.5	0-100	10.3	0-60	10	0-70
P-value *		<0.05		<0.05		<0.05	
Invasive EFVPTC	24	66.2	0-100	80	30-100	55	0-100
P-value **		>0.05		>0.05		>0.05	

NOTE. The *P*-value assesses the differences in the extent of the three parameters between NIFTP and benign follicular lesions (*) and between NIFTP and invasive EFVPTC (**)

Table 4. Correlation between nuclear score and categorization according to the Bethesda System in different tumor types.

Tumor type (N of cases)	**Nuclear Score (N of cases)**	**Categorization according to Bethesda System**			
		III	**IV**	**V**	**VI**
NIFTP (96)	Score 0-1 (22)	12	9	1	0
	Score 2-3 (74)	2	45	25	2
Benign follicular lesions (37)	Score 0-1 (35)	16	19	0	0
	Score 2-3 (2)	1	1	0	0
Invasive EFVPTC (24)	Score 0-1 (2)	0	2	0	0
	Score 2-3 (22)	0	13	9	0

NOTE. The chi-square test showed significant correlation between nuclear score and Bethesda categorization in the NIFTP category (Chi-square: 32.999, $P<0.001$)

Table 5. Concordance of scores between FNAB cytology and histology in the whole series of thyroid lesions studied.

		Histology	
		Score 0-1	Score 2-3
Cytology	Score 0-1	35	24
	Score 2-3	2	96
Cohen's kappa		0.62 (IC 95% 0.49-0.74)	